

WHAT IS CLAIMED IS:

1           1.     An isolated nucleic acid that encodes an ADNF III polypeptide,  
2 wherein said ADNF III polypeptide specifically binds to a polyclonal antibody generated  
3 against an immunogen comprising an amino acid sequence selected from the group  
4 consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:55, SEQ ID NO:57, and SEQ  
5 ID NO:59 and conservatively modified variations thereof.

1           2.     The isolated nucleic acid in accordance with claim 1, wherein said  
2 ADNF III polypeptide has an amino acid sequence selected from the group consisting of  
3 SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:55, SEQ ID NO:57, and SEQ ID NO:59  
4 and conservatively modified variations thereof.

1           3.     An isolated nucleic acid that encodes an ADNF III polypeptide,  
2 wherein said isolated nucleic acid specifically hybridizes, under stringent conditions, to  
3 an *ADNF III* gene in the presence of a human genomic library, said *ADNF III* gene  
4 having a nucleic acid sequence comprising SEQ ID NO:2, SEQ ID NO:56, or SEQ ID  
5 NO:58.

1           4.     The isolated nucleic acid in accordance with claim 1, wherein said  
2 isolated nucleic acid has a nucleic acid sequence comprising SEQ ID NO:2, SEQ ID  
3 NO:56, or SEQ ID NO:58.

1           5.     The isolated nucleic acid in accordance with claim 1, wherein said  
2 isolated nucleic acid specifically hybridizes, under stringent conditions, to an *ADNF III*  
3 gene in the presence of a murine genomic library, said *ADNF III* gene having a nucleic  
4 acid sequence comprising SEQ ID NO:4 or SEQ ID NO:54.

1           6.     The isolated nucleic acid in accordance with claim 1, wherein said  
2 isolated nucleic acid has a nucleic acid sequence comprising SEQ ID NO:4 or SEQ ID  
3 NO:54.

1                   7. An isolated nucleic acid encoding an ADNF III polypeptide  
2 comprising at least 10 contiguous amino acids from a polypeptide having an amino acid  
3 sequence comprising SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:55, SEQ ID NO:57,  
4 and SEQ ID NO:59 and conservatively modified variations thereof, wherein:

5                   said ADNF III polypeptide, when presented as an immunogen, elicits the  
6                   production of an antibody that specifically binds to a polypeptide  
7                   having an amino acid sequence selected from the group consisting  
8                   of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:55, SEQ ID NO:57,  
9                   and SEQ ID NO:59; and

10                  said ADNF III polypeptide does not bind to antisera raised against a  
11                  polypeptide having an amino acid sequence selected from the group  
12                  consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:55, SEQ  
13                  ID NO:57, and SEQ ID NO:59, which has been fully  
14                  immunosorbed with a polypeptide having an amino acid sequence  
15                  comprising SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:55, SEQ  
16                  ID NO:57, and SEQ ID NO:59.

1                   8. The nucleic acid in accordance with claim 7, wherein said nucleic  
2 acid specifically hybridizes to a clone of a human *ADNF III* gene present in a human  
3 genomic library under stringent conditions.

1                   9. The isolated nucleic acid in accordance with claim 7, wherein said  
2 nucleic acid further comprises a recombinant vector.

1                   10. An isolated nucleic acid that encodes an ADNF III polypeptide,  
2 wherein the nucleic acid is amplified by primers that specifically hybridize under  
3 stringent hybridization conditions to the same sequence as a primer set comprising  
4 primers selected from the group consisting of:

5                   sense 5' TCCAATGTTCACCTGCAG 3' (SEQ ID NO:7);  
6                   sense 5' ACCTGCAGCAAAACAACAT 3' (SEQ ID NO:9) and  
7                   antisense 5' GCTCGTTACAGATTGTAC 3' (SEQ ID NO:8).

1                   11. An isolated nucleic acid, which nucleic acid is an ADNF III  
2 promoter, wherein said isolated nucleic acid specifically hybridizes, under stringent  
3 conditions, to an *ADNF III* gene in the presence of a human genomic library, said *ADNF*  
4 *III* gene having a nucleic acid sequence comprising SEQ ID NO:60.

1                   12. An isolated ADNF III polypeptide, said ADNF III polypeptide  
2 specifically binding to an antibody generated against an immunogen having an amino acid  
3 sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID  
4 NO:55, SEQ ID NO:57, and SEQ ID NO:59 and conservatively modified variations  
5 thereof.

1                   13. The isolated ADNF III polypeptide in accordance with claim 12,  
2 wherein said ADNF III polypeptide is human.

1                   14. The isolated ADNF III polypeptide in accordance with claim 12,  
2 wherein said ADNF III polypeptide has an amino acid sequence comprising SEQ ID  
3 NO:1, SEQ ID NO:57 or SEQ ID NO:59.

1                   15. The isolated ADNF III polypeptide in accordance with claim 12,  
2 wherein said ADNF III polypeptide is murine.

1                   16. The isolated ADNF III polypeptide in accordance with claim 12,  
2 wherein said ADNF III polypeptide has an amino acid sequence comprising SEQ ID  
3 NO:3 or SEQ ID NO:55.

1                   17. An isolated ADNF III polypeptide, said ADNF III polypeptide  
2 comprising a subsequence of at least 8 contiguous amino acids of a polypeptide having an  
3 amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID  
4 NO:3, SEQ ID NO:55, SEQ ID NO:57, and SEQ ID NO:59 and conservatively modified  
5 variations thereof.

1                   18. The isolated ADNF III polypeptide in accordance with claim 17,  
2 wherein said ADNF III polypeptide comprising a subsequence of at least 50 contiguous

amino acids of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:55, SEQ ID NO:57, and SEQ ID NO:59 and conservatively modified variations thereof.

19. An isolated ADNF III polypeptide comprising at least 8 contiguous amino acids from a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:55, SEQ ID NO:57, and SEQ ID NO:59 and conservatively modified variations thereof, wherein:

said ADNF III polypeptide, when presented as an immunogen, elicits the production of an antibody that specifically binds to a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:55, SEQ ID NO:57, and SEQ ID NO:59 and conservatively modified variations thereof; and

said ADNF III polypeptide does not bind to antisera raised against a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:55, SEQ ID NO:57, and SEQ ID NO:59 and conservatively modified variations thereof, which has been fully immunosorbed with a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:55, SEQ ID NO:57, and SEQ ID NO:59 and conservatively modified variations thereof.

20. The isolated ADNF III polypeptide of claim 19, wherein said ADNF III polypeptide is encoded by a nucleic acid having a nucleic acid sequence comprising SEQ ID NO:2, SEQ ID NO:56, or SEQ ID NO:58.

21. The isolated ADNF III polypeptide of claim 19, wherein said ADNF III polypeptide is recombinantly reproduced.

22. An antibody that specifically binds to an ADNF III polypeptide comprising at least 8 contiguous amino acids from a polypeptide having an amino acid

sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:55, SEQ ID NO:57, and SEQ ID NO:59 and conservatively modified variations thereof, wherein:

said ADNF III polypeptide, when presented as an immunogen, elicits the production of an antibody that specifically binds to a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:55, SEQ ID NO:57, and SEQ ID NO:59 and conservatively modified variations thereof; and

said ADNF III polypeptide does not bind to antisera raised against a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:55, SEQ ID NO:57, and SEQ ID NO:59 and conservatively modified variations thereof, which has been fully immunosorbed with a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:55, SEQ ID NO:57, and SEQ ID NO:59 and conservatively modified variations thereof.

23. The antibody in accordance with claim 22, wherein said antibody is monoclonal.

24. A recombinant cell comprising a nucleic acid encoding an ADNF III polypeptide comprising at least 8 contiguous amino acids from a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:55, SEQ ID NO:57, and SEQ ID NO:59 and conservatively modified variations thereof, wherein:

said ADNF III polypeptide, when presented as an immunogen, elicits the production of an antibody that specifically binds to a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:55, SEQ ID NO:57, and SEQ ID NO:59 and conservatively modified variations thereof; and

said ADNF III polypeptide does not bind to antisera raised against a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:55, SEQ ID NO:57, and SEQ ID NO:59 and conservatively modified variations thereof, which has been fully immunosorbed with a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:55, SEQ ID NO:57, and SEQ ID NO:59 and conservatively modified variations thereof.

**25. An Activity Dependent Neurotrophic Factor (ADNF) III**

polypeptide, said polypeptide comprising the following amino acid sequence:

$(R^1)_x$ -Asn-Ala-Pro-Val-Ser-Ile-Pro-Gln- $(R^2)_y$  (SEQ ID NO:10)

and conservatively modified variations thereof, in which:

$R^1$  is an amino acid sequence comprising from 1 to about 40 amino acids wherein each amino acid is independently selected from the group consisting of naturally occurring amino acids and amino acid analogs;

$R^2$  is an amino acid sequence comprising from 1 to about 40 amino acids wherein each amino acid is independently selected from the group consisting of naturally occurring amino acids and amino acid analogs; and

x and y are independently selected and are equal to zero or one.

**26. The ADNF III polypeptide in accordance with claim 25 wherein:**

x and y are both zero.

**27. The ADNF III polypeptide in accordance with claim 25 wherein:**

x is one;

$R^1$  is Gly-Gly-; and

y is zero.

1           28. The ADNF III polypeptide in accordance with claim 25 wherein:

2           x is one;

3           R<sup>1</sup> is Leu-Gly-Gly-;

4           y is one; and

5           R<sup>2</sup> is -Gln-Ser.

1           29. The ADNF III polypeptide in accordance with claim 25 wherein:

2           x is one;

3           R<sup>1</sup> is Leu-Gly-Leu-Gly-Gly- (SEQ ID NO:17);

4           y is one; and

5           R<sup>2</sup> is -Gln-Ser.

1           30. The ADNF III polypeptide in accordance with claim 25 wherein:

2           x is one;

3           R<sup>1</sup> is Ser-Val-Arg-Leu-Gly-Leu-Gly-Gly- (SEQ ID NO:18);

4           y is one; and

5           R<sup>2</sup> is -Gln-Ser.

1           31. A method for preventing neuronal cell death, said method

2           comprising contacting said neuronal cells with an Activity Dependent Neurotrophic

3           Factor (ADNF) III polypeptide in an amount sufficient to prevent neuronal cell death.

1           32. The method in accordance with claim 31, wherein said neuronal

2           cells are selected from the group consisting of spinal cord neurons, hippocampal neurons,

3           cerebral cortical neurons and cholinergic neurons.

1           33. The method in accordance with claim 31, wherein said ADNF III

2           polypeptide has an amino acid sequence selected from the group consisting of SEQ ID

3           NO:1, SEQ ID NO:3, SEQ ID NO:55, SEQ ID NO:57, and SEQ ID NO:59 and

4           conservatively modified variations thereof.

1           34. The method in accordance with claim 31, wherein said ADNF III

2           polypeptide comprises the following amino acid sequence:

(R<sup>1</sup>)<sub>x</sub>-Asn-Ala-Pro-Val-Ser-Ile-Pro-Gln-(R<sup>2</sup>)<sub>y</sub> (SEQ ID NO:10)

and conservatively modified variations thereof, in which:

R<sup>1</sup> is an amino acid sequence comprising from 1 to about 40 amino acids wherein each amino acid is independently selected from the group consisting of naturally occurring amino acids and amino acid analogs;

R<sup>2</sup> is an amino acid sequence comprising from 1 to about 40 amino acids wherein each amino acid is independently selected from the group consisting of naturally occurring amino acids and amino acid analogs; and

x and y are independently selected and are equal to zero or one.

35. The method in accordance with claim 34, wherein:

x and y are both zero.

36. A method for preventing neuronal cell death in a patient infected with human immunodeficiency virus, said method comprising administering to said patient an Activity Dependent Neurotrophic Factor (ADNF) III polypeptide in an amount sufficient to prevent neuronal cell death and a pharmaceutically acceptable carrier.

37. The method in accordance with claim 36 wherein said ADNF III polypeptide has an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:55, SEQ ID NO:57, and SEQ ID NO:59 and conservatively modified variations thereof.

38. The method in accordance with claim 36 wherein said ADNF III polypeptide comprises the following amino acid sequence:

(R<sup>1</sup>)<sub>x</sub>-Asn-Ala-Pro-Val-Ser-Ile-Pro-Gln-(R<sup>2</sup>)<sub>y</sub> (SEQ ID NO:10)

and conservatively modified variations thereof, in which:

R<sup>1</sup> is an amino acid sequence comprising from 1 to about 40 amino acids wherein each amino acid is independently selected from the group



consisting of naturally occurring amino acids and amino acid  
analog;

$R^2$  is an amino acid sequence comprising from 1 to about 40 amino acids  
wherein each amino acid is independently selected from the group  
consisting of naturally occurring amino acids and amino acid  
analog; and

$x$  and  $y$  are independently selected and are equal to zero or one.

39. The method in accordance with claim 38, wherein:

$x$  and  $y$  are both zero.

40. A method for preventing neuronal cell death associated with  
excito-toxicity induced by *N*-methyl-D-aspartate stimulation, said method comprising  
contacting said neuronal cells with an Activity Dependent Neurotrophic Factor (ADNF)  
III polypeptide in an amount sufficient to prevent neuronal cell death.

41. The method in accordance with claim 40, wherein said ADNF III  
polypeptide has an amino acid sequence selected from the group consisting of SEQ ID  
NO:1, SEQ ID NO:3, SEQ ID NO:55, SEQ ID NO:57, and SEQ ID NO:59 and  
conservatively modified variations thereof.

42. The method in accordance with claim 40, wherein said ADNF III  
polypeptide comprises the following amino acid sequence:

$(R^1)_x$ -Asn-Ala-Pro-Val-Ser-Ile-Pro-Gln- $(R^2)_y$  (SEQ ID NO:10)

and conservatively modified variations thereof, in which:

$R^1$  is an amino acid sequence comprising from 1 to about 40 amino acids  
wherein each amino acid is independently selected from the group  
consisting of naturally occurring amino acids and amino acid  
analog;

$R^2$  is an amino acid sequence comprising from 1 to about 40 amino acids  
wherein each amino acid is independently selected from the group

consisting of naturally occurring amino acids and amino acid  
analog; and  
x and y are independently selected and are equal to zero or one.

43. The method in accordance with claim 42, wherein:  
x and y are both zero.

44. A method of preventing neuronal cell death induced by the  $\beta$ -  
amyloid peptide in a patient afflicted with Alzheimer's disease, said method comprising  
administering to said patient an Activity Dependent Neurotrophic Factor (ADNF) III  
polypeptide in an amount sufficient to prevent neuronal cell death and a pharmaceutically  
acceptable carrier.

45. The method in accordance with claim 44, wherein said ADNF III  
polypeptide has an amino acid sequence selected from the group consisting of SEQ ID  
NO:1, SEQ ID NO:3, SEQ ID NO:55, SEQ ID NO:57, and SEQ ID NO:59 and  
conservatively modified variations thereof.

46. The method in accordance with claim 44, wherein said ADNF III  
polypeptide comprises the following amino acid sequence:

$(R^1)_x$ -Asn-Ala-Pro-Val-Ser-Ile-Pro-Gln- $(R^2)_y$  (SEQ ID NO:10)

and conservatively modified variations thereof, in which:

$R^1$  is an amino acid sequence comprising from 1 to about 40 amino acids  
wherein each amino acid is independently selected from the group  
consisting of naturally occurring amino acids and amino acid  
analog; and

$R^2$  is an amino acid sequence comprising from 1 to about 40 amino acids  
wherein each amino acid is independently selected from the group  
consisting of naturally occurring amino acids and amino acid  
analog; and

x and y are independently selected and are equal to zero or one.

14                   47. The method in accordance with claim 46, wherein:

15                   x and y are both zero.

1                   48. A method of alleviating learning impairment produced by

2 cholinergic blockage in a patient afflicted with Alzheimer's disease, said method  
3 comprising administering to said patient an Activity Dependent Neurotrophic Factor  
4 (ADNF) III polypeptide in an amount sufficient to prevent neuronal cell death and a  
5 pharmaceutically acceptable carrier.

1                   49. The method in accordance with claim 48, wherein said ADNF III

2 polypeptide has an amino acid sequence selected from the group consisting of SEQ ID  
3 NO:1, SEQ ID NO:3, SEQ ID NO:55, SEQ ID NO:57, and SEQ ID NO:59 and  
4 conservatively modified variations thereof.

1                   50. The method in accordance with claim 48, wherein said ADNF III

2 polypeptide comprises the following amino acid sequence:

3                    $(R^1)_x$ -Asn-Ala-Pro-Val-Ser-Ile-Pro-Gln- $(R^2)_y$  (SEQ ID NO:10)

4 and conservatively modified variations thereof, in which:

5                   R<sup>1</sup> is an amino acid sequence comprising from 1 to about 40 amino acids  
6                   wherein each amino acid is independently selected from the group  
7                   consisting of naturally occurring amino acids and amino acid  
8                   analogs;

9                   R<sup>2</sup> is an amino acid sequence comprising from 1 to about 40 amino acids  
10                  wherein each amino acid is independently selected from the group  
11                  consisting of naturally occurring amino acids and amino acid  
12                  analogs; and

13                  x and y are independently selected and are equal to zero or one.

1                   51. The method in accordance with claim 50, wherein:

2                   x and y are both zero.

1                   52. A pharmaceutical composition comprising a pharmaceutically  
2 acceptable excipient and an Activity Dependent Neurotrophic Factor (ADNF) III  
3 polypeptide.

1                   53. The pharmaceutical composition in accordance with claim 52,  
2 wherein said ADNF III polypeptide has an amino acid sequence selected from the group  
3 consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:55, SEQ ID NO:57, and SEQ  
4 ID NO:59 and conservatively modified variations thereof.

1                   54. The pharmaceutical composition in accordance with claim 52,  
2 wherein said ADNF III polypeptide comprises the following amino acid sequence:

3                                $(R^1)_x$ -Asn-Ala-Pro-Val-Ser-Ile-Pro-Gln- $(R^2)_y$  (SEQ ID NO:10)

4 and conservatively modified variations thereof, in which:

5                    $R^1$  is an amino acid sequence comprising from 1 to about 40 amino acids  
6                               wherein each amino acid is independently selected from the group  
7                               consisting of naturally occurring amino acids and amino acid  
8                               analogs;

9                    $R^2$  is an amino acid sequence comprising from 1 to about 40 amino acids  
10                              wherein each amino acid is independently selected from the group  
11                              consisting of naturally occurring amino acids and amino acid  
12                              analogs; and

13                   x and y are independently selected and are equal to zero or one.

1                   55. The pharmaceutical composition in accordance with claim 54,  
2 wherein:

3                   x and y are both zero.